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Ceva Animal Health Asia Pacific

HATCHERY
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MICROBIOLOGICAL TOOLS FOR QUALITY ASSURANCE IN HATCHERY :

Sampling Procedures

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Any sanitation program should be assessed routinely for checking its stable and continuous efficacy and, if necessary, its improvement. Indeed, the eggs and the day-old-chicks can be contaminated in the breeder house itself, during the egg holding procedures or in the hatchery itself. This routine monitoring should focus at least on air and surface sampling. The regular recording of the generated data will then allow a better follow up and diagnostic in case of troubleshooting. In case of any particular that needs to be solved, a deeper and more precise monitoring procedure can be applied, including egg washes, chick sampling and bacterial identification.

BEFORE COMMENCING THE ENVIRONMENTAL SAMPLING

The person in charge of the monitoring procedures should take the following steps:

- ☑ Draw a process flow chart and hatchery traffic pattern;
- ☑ Determine target organism(s) or hazards;
- ☑ Identify the critical control points;
- ☑ Determine sampling sites;
- ☑ Arrange with laboratory personnel for sterile equipment and sterile material;
- ☑ Identify material and record according to sampling sites.

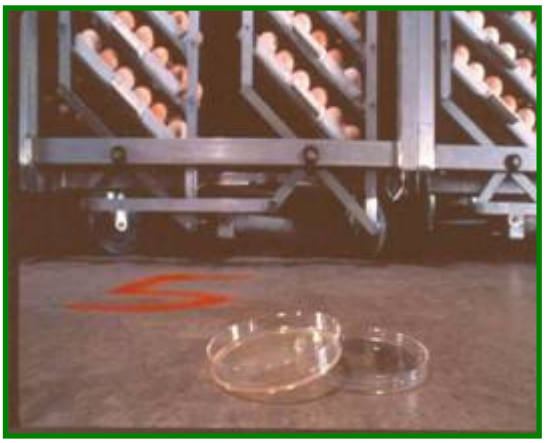
If information is needed regarding cleaning and sanitation efficacy in the hatchery, it is recommended that samples are taken immediately just after cleaning and sanitation have been completed.

If information is needed regarding the general microbiological environmental conditions and biosecurity barriers efficiency, it is recommended that samples are not taken right after disinfection.

HOW TO SAMPLE HATCHERY AIR?

There are two major procedures:

- The most commonly used is the “air plate”. Areas concerned by this method are mainly hatchery hallways, egg rooms, chick rooms, setters and hatchers. The bottom of the plate is marked with the location to be monitored. The Petri plate with the selected media is exposed by carefully placing the plate (media on the bottom) on a flat surface within the environment to be monitored and gently removing the cover, letting it rest on a clean surface. The length of time that the media is exposed to the air will depend upon the expected contamination level of the area. It is extremely important that the length of exposure is consistent when sampling the air in a particular environment from one time to the next to allow meaningful comparisons. If the area is a relatively clean environment such as a setter, hallway or cleaned hatcher, a ten-minute sampling time is suggested.



- Mechanical air sampling devices (such as the Anderson Air Sampler / RCS unit or the OMNI3000 portable machine – cf. picture below) are generally battery powered and allow a set volume of air to pass over a set of agar wells which allows the operator to assess the contamination level. The standard time is one minute. The type of agar media in the wells can vary depending on the sampling material.

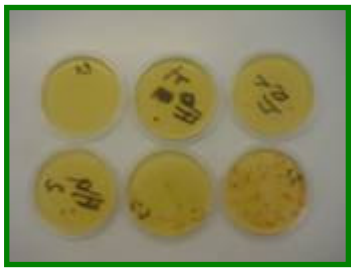
HOW TO SAMPLE FLAT SURFACES?

This method is interesting for monitoring after cleaning and drying incubator walls, farm racks, cases or fan blades. As usual, all plates must be marked on the bottom (the half plate containing the media) indicating the type and location of surface being monitored.

- The swab method may be used to sample any surface up to one or two square inches. Cloths would be preferred for walls and floors ($1m^2$), and fabric-tipped swabs and sponges for equipment and other small contact surfaces ($100cm^2$). A sterile swab which has been moistened in a sterile solution or a manufactured sterile culturette may be rubbed gently over a predefined area of the sample surface. The swab is then gently streaked over the surface of the plate several times in a zigzag fashion. If the sample has to be transported, there should be one sterile premoistened swab, cloth or sponge per container. No residual diluents should be present in the container.

***Note**: It can be a good practice to have two swabs as controls. One left unopened and one transferred to the media without being used to swab.*

- Another method of sampling surfaces employs the RODAC contact plate (*Replicate Organism Direct Agar Contact*). Actually, their use is recommended for sampling flat, impervious surfaces; the use of Petrifilm™ is recommended for sampling irregular surfaces.



→ Rodac Plate Method *(surface area covered by a RODAC plate is approximately 25 cm^2)*

- Wearing sterile gloves, open the bag containing RODAC plates and pick one RODAC plate. RODAC plates should be made so that the agar is slightly higher than the edge of the plate.
- Remove plastic cover and carefully press agar surface to the surface being sampled.
- Make sure that the entire agar surface contacts the area to be sampled by applying uniform pressure on the back of the plate.
- Replace cover. Pile up used plates (5 to 10) and secure them with masking tape.
- Put plates in Whirlpak™ bags or other suitable containers.
- Seal bag tightly and handle carefully during transport to laboratory.

Note: Because of concerns by hatchery management of possible contamination, the surface sampled should be wiped with a sterile wet cloth or a sanitizer (e.g. 70% ethanol) before going to the next sampling site.

→ Petrifilm™ [10] Method *(surface area covered by a RODAC plate is approximately 20 cm^2)*

- Petrifilm plates must be rehydrated by laboratory personnel prior to use.
- Wearing sterile gloves, lift off the top film of the Petrifilm™ plate (gel will adhere to top film).
- The gel (on top film, not bottom part) must be put in contact to the surface being tested.
- Avoid touching the growth area and bottom cardboard with fingers or food contact surfaces not being sampled.
- Firmly rub fingers over the entire film side of the gelled area to ensure good contact with surface.
- Lift film from surface and rejoin the top and bottom sheets of the Petrifilm™.

Note: On occasion the gel may split (adhering to both the top and bottom of the Petrifilm™) when the top film is lifted. This splitting of the gel will not affect the performance of the product and it should be used as described above.

HOW TO SAMPLE LIQUIDS?

Sterile agar plate must be poured with 0.5 to 1 ml of the liquid to be tested. Then, a sterile swab or a sterile "L" shaped glass rod can be used to spread this aliquot onto the medium. The excess moisture on it should be allowed to dry by incubating it for two hours. It is obviously necessary to label the bottom of the plate to identify the substance being monitored.

HOW TO SAMPLE FLUFF AND OTHER SOLIDS?

The first step, after weighing aseptically a particular amount of the solid to analyze, is to dilute it ten times in sterile saline. A very homogenous solution must be then got by strongly mixing by hand or with a Vortex for at least 30 sec. (1 ml of the mother solution placed into 9 ml of sterile saline for 10% dilution).

The same procedure will be applied to get 0.01%, 0.1%, 1% solutions besides this 10% solution. With a sterile 'L' shaped glass rod, 1 ml of each diluted solution should then be pipetted and spread on the media surface. Same as aforementioned, the media soaked in this way should be dried for a couple of hours in the incubator with the media at the bottom before putting it in the normal position.

SAMPLING EGGSHELL SURFACES

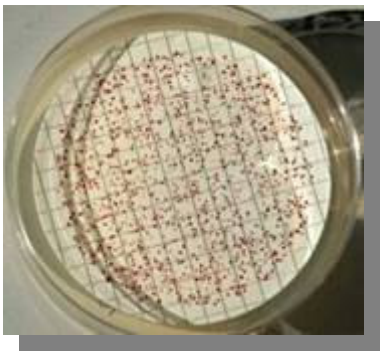
QUALITY	BACTERIA PER EGG
Freshly laid eggs	300+ colonies
Clean eggs	3,000+ colonies
Soiled eggs	25,000+ colonies
Dirty eggs	400,000+ colonies

The surfaces of eggs laid in farms are obviously not sterile and this is all the purpose of egg disinfection with chemicals (like formalin) in the farms and at arrival in hatchery to reduce the contamination as much as possible.
An eggshell surface monitoring is consequently a very good help for checking and adjusting the procedure, or it can also be used whenever a contamination problem has been identified by the egg breakout program.

1) A common method of monitoring the shell surface involves rolling the egg surface on a plate. To perform this method, the plate cover should be removed and placed on a clean surface. The egg should be picked up with your fingertips and the surface which is untouched by your fingers rolled on the surface of the media. Eggs should be rolled in this fashion two times and the cover of the plate should be carefully replaced. This method works as a screening test, but does not provide as accurate a microbial count as the methods described here below.

2) A more quantitative method for determining the contamination level of eggshell surfaces is the "total egg wash". After picking up the egg with a clean tissue, place it in a sterile whirl-pack bag containing 20 ml of sterile physiological saline. The egg should be then gently massaged in the bag for one minute, let stand for one minute and massage again for one minute. Caution should be taken not to contaminate further the wash water when removing the egg.

The mold or the bacterial enumeration will be then assessed by inoculating aliquots of 0.1 ml and 1 ml on media plates with relevant media. As usual, egg wash water should be spread with a sterile swab or a sterile glass "L" rod and the plates should be incubated for two hours with the media on the bottom to allow the excess moisture to soak into the media. It is necessary to bear in mind that, for routine monitoring, the identification of specific microorganisms is less important than the general trend of groups of micro-organisms and changes over time in shell surface contamination. The colonies on the plates should be counted, corrected for dilution factor (1:20 and 1:200), and recorded.



3) Strips of sterile tape can also be used instead of the aforementioned methods. The tape (# 1.8 cm x 1.6 cm) should be applied to the shell surface, carefully removed and then, an impression can be made on an agar plate. Two taping impressions per egg should be adequate for estimating the total number of bacteria on the eggshell. Nevertheless, the total bacteria count using the taping technique tends to be lower than that obtained by using the egg wash technique.

SAMPLING CHICKS

Each breeder flock should be monitored by the analysis of the day-old chicks' microbiological quality. For this purpose, about 10 of them must be culled by dislocating the cervical vertebras.
In order to prevent airborne contamination, the abdomen should be opened only after having soaked the dawn with water and disinfectant. After this procedure, the yolk sac is perforated with a sterile, moist swab. The swab is, then, twirled inside the yolk. The last step of this procedure is to remove the swab soaked with yolk very carefully.

This swab can be streaked on the surface of an agar plate to allow the growth of any germ that could be carried by the sampled chicks. Again to avoid any contamination, the cover of the plate should be carefully replaced immediately after streaking.

CONCLUSION

Evaluating the microbial monitoring program is the most important step. It is necessary to make sure that all results will be recorded and be able to notify any changes occurring over time. Consequently, the sampling procedure must be well planned prior to its implementation and all the steps respected and recorded with relevant identification of the samples. Only then, the results in each monitored area should be compared with hatchability and chick livability data.

In the next issue, the material necessary and the laboratory procedures of this microbiological monitoring will be discussed. Indeed, after sanitization of one given area, the microbiological level for each criteria monitored should be 20 colonies or less. Depending on the hygiene and the structure of the monitored hatchery, targets can be posed in a progression way to achieve this reference after a while. Excessive colonies will indicate poor sanitation procedures or a hatching egg production problem.
Early detection of contamination can minimize hatchery and chick quality problems, which are of utmost importance for the broiler performance at the end of the day.

...every detail matters for performance

CHICK PROGRAM

CEVA HATCHERY INJECTION CONTROL KEYS

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